2

EFFECTS ON FLAX OF A TOXIC CONCENTRATION OF BORON, IRON, MOLYBDENUM, ALUMINIUM, COPPER, ZINC, MANGANESE, COBALT OR NICKEL IN THE NUTRIENT SOLUTION

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Contents

Introduction

During the years 1943 to 1947 inclusive, numerous water culture experiments were conducted to determine the symptoms in flax resulting from the addition to the nutrient solution of excessive amounts of boron, iron, molybdenum, aluminium, copper, zinc, manganese, cobalt or nickel. Interactions of the effects of many of these elements were also studied and the results are described and discussed.

Method

The nutrient solution used was that of Arnon (1938), the composition of which was as follows:

Potassium phosphate	K_2HPO_4 or KH_2PO_4	0·001 Molar
Potassium nitrate	KNO_3	0·006 „
Calcium nitrate	$Ca(NO_3)_2.4H_2O$	0·004 „
Magnesium sulphate	$MgSO_4.7H_2O$	0·002 „
Boron as Boric acid	H_3BO_3	0·5 parts per million
Manganese as manganese sulphate	$MnSO_4.4H_2O$	0·5 „ „ „
Zinc as zinc sulphate	$ZnSO_4.7H_2O$	0·05 „ „ „
Copper as copper sulphate	$CuSO_4.5H_2O$	0·02 „ „ „
Vanadium as Ammonium vanadate	NH_4VO_3	0·01 „ „ „
Chromium as Chrome alum	$Cr_2K_2(SO_4)_4.24H_2O$	0·01 „ „ „
Nickel as nickel sulphate	$NiSO_4.6H_2O$	0·01 „ „ „
Cobalt as cobalt nitrate	$Co(NO_3)_2.6H_2O$	0·01 „ „ „
Tungsten as Sodium tungstate	$Na_2WO_4.2H_2O$	0·01 „ „ „
Molybdenum as Ammonium molybdate	$(NH_4)_2MoO_4$	0·01 „ „ „

Iron was added to the solution in the various experiments as one of the following two forms:

(a) An iron solution containing 0·5 per cent $FeSO_4.7H_2O$ and 0·4 per cent tartaric acid was added twice weekly at the rate of 0·6 ml. per litre of culture solution.

(b) A solution containing 0·5 per cent Ferric citrate was added twice weekly at the rate of 0·5 ml. per litre of culture solution. However, the first of these solutions was the one most commonly used, as it proved to be the more satisfactory source of iron for flax.

In some of the experiments in which the effects of excesses of manganese, zinc, copper and molybdenum were studied, sodium chloride was included in the culture solution at the rate of 0·05 grams per litre.

The general procedure for the water cultures was similar to that described previously (Millikan, 1942, 1943). The flax varieties used were Liral Crown and Concurrent and the seedlings were germinated in washed sand, and transferred as soon as possible after emergence to the water cultures. Two-litre pyrex beakers were used and twelve seedlings were grown in each. The 'excess' treatments were usually applied immediately after setting up the cultures, and the experiments were normally concluded approximately six to eight weeks later. In one experiment, however, the plants were grown for several weeks in a normal solution before being subjected to an excess manganese treatment. All treatments were set up in duplicate.

The following are details of the treatments when an element was applied in excess of its amount in the normal solution:

Boron (as H_3BO_3). In the first experiment excess boron was added at the rate of 2·5 parts per million. After four weeks this was increased to 12·5 p.p.m. Toxicity symptoms began to appear seven days after this adjustment. In a second experiment 4 p.p.m. of boron were added.

Iron. The ferrous sulphate solution described above was used in the various experiments. It was added at 2, 3, 5 or 10 times the normal rate.

Molybdenum (as $(NH_4)_2MoO_4$), 0·1, 0·5, 1, 5, 10, 25, 50 and 100 p.p.m. respectively. The effect of the addition of molybdenum to solutions containing excessive amounts of manganese, zinc, copper, cobalt or nickel is described elsewhere (Millikan, 1947c).

Copper (as $CuSO_4.5H_2O$), 0·05, 0·1, 0·5, 1, 2, 3, 5, 10, 25 and 50 p.p.m. respectively.

Aluminium. A half molar solution of $AlCl_3.6H_2O$ was added at rates of 1, 3 and 6 ml. respectively per litre of culture solution. In other experiments aluminium (as $AlK(SO_4)_2.12H_2O$) was added at the rates of 1, 5, 25, 50 and 100 p.p.m. respectively.

In yet other experiments aluminium was added as $Al_2(SO_4)_3$ at rates of 1, 5, 25 and 50 p.p.m. respectively. In this instance the plants were grown on alternate days in a solution with twice the normal phosphorus and iron contents, and then in a solution without phosphorus and iron but with aluminium. This was to prevent any precipitation of aluminium by the phosphorus in the nutrient solution. The control plants were

grown on alternate days in solutions either containing twice the normal phosphorus and iron contents, or lacking these two elements but without the addition of aluminium.

Cobalt (as $Co(NO_3)_2.6H_2O$), 0·5, 1, 2 and 5 p.p.m. respectively.

Nickel (as $NiSO_4.7H_2O$), 0·5, 1, 2 and 5 p.p.m. respectively.

Zinc (as $ZnSO_4.7H_2O$), 5, 10, 20, 50 and 100 p.p.m respectively.

Manganese (as $MnSO_4.4H_2O$), 10, 20, 25, 50, 100 and 150 p.p.m. respectively.

Zinc and manganese toxicities were each studied in conjunction with varying concentrations of iron, phosphorus, calcium and aluminium respectively in the nutrient solution.

Two nutrient solutions were used in which the source of phosphorus was either K_2HPO_4 (pH 7·0) or KH_2PO_4 (pH 4·5).

Results

The following are details of the results obtained from the water culture experiments:

Excess Boron

The leaves in the middle portion of the stem were first affected, while the youngest leaves were last to show symptoms. These symptoms consisted of a greyish green transparent discoloration which commenced at the tips of the leaves, and gradually extended downwards until the whole leaf was dead. No yellowing of the top leaves occurred.

Eaton (1940), Ferguson and Wright (1940), and Eaton (1944) have also shown that boron toxicity symptoms are first manifest in the older leaves of the plant. Reeve and Shive (1944) have demonstrated that increasing potassium concentrations in the substrate progressively accentuated the symptoms of boron toxicity, whereas an increase in the calcium concentration markedly reduces toxicity symptoms. The Ca/B ratio was shown to determine the response of the tomato plant to boron applications. An increase in the potassium concentration markedly decreased this ratio. Jones and Scarseth (1944) have similarly concluded that for normal plant growth a certain balance between the absorption of calcium and boron is necessary, although the optimum ratio varied for different plant species.

Excess Iron

Growth became very dwarfed, and the whole plant developed a much darker green colour than normal. In the presence of very excessive quantities of iron, the roots were also brownish, very swollen, and much reduced in growth, and with very stunted lateral roots, compared with the controls. The appearance of such roots was similar to those of plants subjected to excess aluminium. Ruprecht (1915) has also noted the similar appearance of the root systems of clover seedlings subjected to iron and aluminium excesses respectively. The symptoms of hyperchlorophylly and restricted growth produced by this treatment appeared to be identical with the symptoms of phosphorus deficiency (McMurtrey

1938, Millikan 1944). By way of confirmation, it was found that excess phosphorus (0·010M or 0·020M $NaH_2PO_4.2H_2O$) added in conjunction with excess iron (3, 5 or 10 times the normal concentration) resulted in a more nearly normal green colour in the plants. In some instances, the tops of the plants actually became chlorotic. Further, the same excess of phosphorus alone caused a chlorosis of the top of the plant which was cured by supplying additional iron to the solution. Olsen (1935), Kapp (1938), Chapman *et al.* (1939), and Chandler and Scarseth (1941) have all demonstrated that excessive phosphate may cause iron deficiency through reactions within the plant.

Further, flax plants receiving excess iron, when phosphorus deficient, were a darker green colour than plants receiving the same excess of iron but a normal supply of phosphorus.

Conversely, in cultures with a deficiency of iron in the solution, it was found that phosphate excess accelerated, and phosphorus deficiency retarded, the development of severe chlorotic symptoms. Controls were flax plants subjected to the same degree of iron deficiency but with a normal phosphorus supply.

Olsen (1935) obtained somewhat similar results. It was shown that in nutrient solutions at pH 6-7 certain plants developed severe chlorosis when ferric chloride was used as a source of iron, but no chlorosis when ferric citrate was used. It was concluded that at pH 6-7 the iron was precipitated in the vascular tissues as phosphate and became unavailable. However, when the amount of phosphate in the nutrient solution was reduced, no chlorosis occurred even when ferric chloride was used.

Additional confirmation of the effect of excess iron in causing phosphorus deficiency in the flax plant was obtained by testing the roots of the plants subjected to the excess iron or phosphorus treatments, respectively, for the presence of inorganically bound phosphorus by the method described by Wright (1945). The results of these tests showed that the roots of the plants receiving an excess of either iron or phosphorus in the nutrient solutions contained abundant inorganically bound phosphorus, whereas little or none was present in the roots of the control plants.

From the above it is concluded that an excess of iron or of phosphorus in the tissues of the plant resulted in some form of antagonistic reaction, thereby causing a deficiency of whichever element is not present in excess.

As a further instance of the relationship between phosphorus and iron in the plant, it was found (as described in the sections on excess manganese and zinc) that phosphorus deficiency considerably delayed the development and reduced the severity of iron deficiency chlorosis, which is normally associated with excessive amounts of manganese or zinc in the nutrient solution.

However, from the literature it is apparent that many factors may upset normal iron metabolism in the plant. Bennett (1945) and Chapman (1945) list the following such factors: Excesses of either phosphorus, potassium (in a solution low in calcium) and manganese, and slight excesses of copper or zinc. Chapman also found that citrus cultures maintained at approximately pH 4·0, and supplied with

adequate iron, developed typical iron chlorosis if subjected to a deficiency of either potassium or magnesium. More iron chlorosis appeared in winter than in summer. This latter observation has been confirmed by Millikan (1945). Thorne and Wallace (1944) have suggested that the balance between ferrous and ferric iron in the plant is very important.

Somers and Shive (1942) and Pearse (1944) have claimed that excessive iron produced a chlorosis which was identical with that induced by lack of manganese, and that the ratio of iron to manganese in the nutrient solution, corresponding to good growth and development, fluctuated within a somewhat narrow range. However, this result could not be confirmed for flax, as none of the plants in the Burnley water cultures, to which considerable excess of iron was added, showed any chlorosis. Kriel (cited by Bennett 1945) has shown that the iron to manganese ratio *in the plant* had no relation to the chlorosis produced.

Further, in unpublished field experiments by the writer, iron citrate applied to flax at the rate of 5 cwt. per acre caused no yellowing, whereas 4 cwt. per acre of manganese sulphate did cause chlorosis. Demetriados (1938) also studied the effects of additions of excessive amounts of iron to the soil upon three different species of plants. The treatments resulted in an increased iron concentration in the tissues, but no chlorosis was observed, although various other toxic effects occurred. Similarly, Scholz (1937) found that in the presence of excessive amounts of iron or of aluminium in the soil, the plant may suffer from phosphate deficiency.

Excess Molybdenum

First symptoms resulting from the presence of excess molybdenum in the solutions were observable in the meristematic tissues of the roots of the plants after three days. These roots assumed a golden-orange colouration, and at the higher concentrations of molybdenum practically no lateral root development occurred, and the main root remained very dwarfed. Warrington (1946) has produced similar root symptoms in lettuce subjected to molybdenum poisoning. With as little as 0·5 p.p.m. of molybdenum in the culture solution, root development of the flax seedlings was restricted and a golden-orange colouration was noticeable after three days. Later, at the lower molybdenum concentrations, lateral root development recovered, and the new roots were not obviously orange in colour.

Top growth was slightly reduced at the lower levels of molybdenum (up to 5 p.p.m.), but at the higher levels (25, 50 and 100 p.p.m.) aerial growth was very severely reduced and a golden-yellow colouration of the leaves occurred. This colouration was most marked at the highest molybdenum concentrations. At lower molybdenum concentrations only the bottom leaves of the plant were usually involved. This golden-yellow colouration was not the same in appearance as the pale yellow to whitish colouration of the top leaves typical of iron deficiency, nor was it prevented by the application of additional iron to the solution.

Warrington (1937, 1946) has also described the occurrence of a golden-yellow or reddish-yellow colour in the shoots of tomato, the tubers of potato, and leaves of lettuce, respectively, when the plants were

grown in solutions containing high concentrations of sodium molybdate. The colour reactions were associated with the presence of yellow globules of a tannin-molybdenum compound in the tissues. Tissues of molybdenum-treated plants containing anthocyanin also showed large numbers of blue granular accumulations which were apparently of an anthocyanin-molybdenum nature.

In view of the evident similarity of the golden-yellow colouration in the flax plants to that described by Warrington, and its apparent dissimilarity to iron deficiency chlorosis, tests for tannins were made on the leaves and roots. The following two reagents were used:

(1) 10% ferric chloride plus a small amount of sodium acetate.
(2) 1% osmic acid.

The formation of a greenish-black colouration with (1), and a black colouration with (2), are positive indications for the presence of tannins. The presence of tannins in the molybdenum poisoned flax tissues was confirmed by these tests.

The important effect of molybdenum in reducing the severity of toxicity symptoms caused by an excess of manganese, zinc, copper, nickel or cobalt has been described elsewhere (Millikan, 1947).

Excess Aluminium

In the various experiments, the symptoms produced by aluminium toxicity were identical, irrespective of whether the excess aluminium was applied in solutions with or without phosphate, indicating that even in the presence of phosphate (which precipitates aluminium) the plant was able to absorb sufficient aluminium to cause poisoning. However, when the aluminium was applied in a separate solution from that of the phosphate, the toxic concentration was less than when aluminium and phosphate were present in the same solution. McLean and Gilbert (1928) have shown that even non-diffusible colloidal aluminium hydroxide in contact with barley roots is definitely harmful.

The addition of aluminium chloride (0·005M) to the culture solution originally pH 7, stimulated the rate of growth of the flax, which became darker green than the normal. No necrotic lesions occurred on the leaves.

In the solution originally pH 4·5, the addition of aluminium was harmful to growth, the injury being first noticeable in the roots three days after setting up the cultures. The plants became dwarfed due to a shortening of the internodes between leaves and a reduction in leaf areas. The leaves were typically darker green than normal but they showed no necrosis. Eisenmenger (1935) has described a similar darkening and dwarfing of the foliage of tobacco plants supplied with aluminium salts. On the other hand, Ligon and Pierre (1932) state that in their cultures containing excess aluminium the leaves of sorghum showed a characteristic chlorosis. However, the arrangements for supplying iron to the test plants in their experiments appear to have been quite inadequate.

The root development in the flax plants receiving excess aluminium in the Burnley experiments was very poor, as little as 5 p.p.m. of

aluminium causing a noticeable reduction in root development, but little or no reduction in aerial growth, while one part per million of aluminium caused no noticeable reduction in root growth. With concentrations of aluminium higher than 5 p.p.m., practically no lateral root development occurred, such roots remaining as short abortive stubs close to the single main primary root, which did not elongate much but became light brown and very swollen and distorted. Similar root characteristics resulting from excess aluminium have been described by Ruprecht (1915), Stoklasa (1919), Magistad (1925), McLean and Gilbert (1927), Ligon and Pierre (1932), and Bortner (1935). The latter has also referred to a slight tip burn on the lower leaves of tobacco plants treated with 4 to 6 parts per million of aluminum. However, in the presence of a concentration of aluminium as high as 100 p.p.m. no leaf necrosis was observed by the writer in flax after one month.

The *direct* toxic effects of aluminium appear to be confined to the root tissues. Ruprecht (1915) found that the stunting of the roots was due to the killing of the cells in the meristematic tissues. Also McLean and Gilbert (1927) showed that aluminium absorbed by the plants accumulated in the cortical tissues of the roots, and was concentrated in the nuclei. However, Peterburgskii (1945) has stated that aluminium ions are mobile in flax plants, which therefore suffer more from aluminium poisoning than do species such as peas which retain most of the aluminium ions in the roots.

The following microchemical tests were made on the roots of plants subjected to excess aluminium in the Burnley water cultures, to confirm that aluminium was actually present in the tissues and to determine its location. The roots to be examined were fixed in formalin acetic alcohol, dehydrated in alcohol, cleared in xylol, embedded in paraffin, sectioned with a microtome and fixed to glass slides with Haupt's adhesive. Roots from both normal solutions and excess-aluminium solutions were included.

(a) *Haematoxylon method* (Hoffer and Carr (1923), Snell (1941)).

1. The tissues were first tested for the presence of iron by being placed in a 20% solution of potassium thiocyanate strongly acidified with hydrochloric acid. This gave a negative reaction.

2. Sections were then placed in a saturated solution of ammonium carbonate which had been coloured deep red by haematoxylon. Lavender blue staining areas indicative of the presence of aluminium occurred in the epidermal cells and cortical tissues immediately underneath. The meristematic tissues of the abortive lateral roots were particularly deeply stained (Pl. II, fig. 1). The colour changed to yellowish brown on the addition of dilute acid. This confirmed the poisoning of the cells in the meristematic region described by Ruprecht (1915).

(b) *Aurin tricarboxylic acid method* (Lange, 1944)

A hot 0·1% aqueous solution of aurin tricarboxylic acid containing a few drops of hydrochloric acid and buffered with ammonium acetate was used. After treatment in this solution, the sections were transferred

to a saturated ammonium carbonate solution to decolourize the excess reagent. The presence of aluminium was indicated by the occurrence of a bright red colour in the epidermal cells and immediately underlying cortical cells (see Plate II, fig. 1).

(c) *Sodium alizarin sulphonate* (*Alizarin Red S*) (Yoshi and Jimbo (1932) and Lange (1944)).

A slightly acidified 0·1% aqueous solution of alizarin red S was used. This produced a bright red lake in the same tissues as the above two reagents. However, all the cells in the root took up the colour to some extent. None of the cells in the roots from the normal solutions was so stained.

A combination of aluminium with phosphate in the plant has been indicated by Sergeev and Sergeev (1929), Pierre and Stuart (1933) and by Wright (1943, 1945). The latter demonstrated the presence of abundant inorganically bound phosphorus in the tissues of the roots in contact with aluminium, but little or none in those from culture solutions lacking this element.

The tests specified by Wright (1945) were made on fresh sections of the roots of the flax plants receiving excess aluminium in the Burnley experiments. These tests also revealed abundant inorganically bound phosphorus in the roots in contact with the aluminium, whereas very little was found to be present in roots from the control plants. As the bound phosphorus was unavailable for plant metabolism, it follows that the abnormally dark green colour of the leaves, and the restricted growth of the flax plants receiving aluminium, were probably symptoms of phosphorus deficiency. McMurtrey (1938) and Millikan (1944) have shown that phosphorus deficient tobacco and flax plants, respectively, manifest similar symptoms.

From the above, it would be expected that the presence of aluminium would increase the phosphorus requirement of plants. Haas (1936b) has found this to be so with citrus, which showed a greatly increased phosphorus content in the presence of aluminium. Wright (1943) also demonstrated that barley plants grown in the presence of aluminium contained a greater percentage of phosporus than did normal plants. However, the water-soluble phosphorus content of the aluminium-treated plants was low, which fact was attributed to the precipitation of phosphorus by aluminium within the plant, thus causing a phosphorus deficiency in the meristematic regions. Previously Hoffer and Carr (1923) had similarly observed that the phosphorus content of the nodal tissues of corn plants was higher when iron and aluminium compounds became concentrated in these tissues.

Further, it has been demonstrated in the Burnley experiments, and also by Conner and Sears (1922) and McLean and Gilbert (1928), that the toxicity of aluminium salts is somewhat counteracted by increasing the amount of phosphate used. This counteraction is evidently due to the provision of sufficient phosphate for plant metabolism in addition to that required to precipitate the aluminium within the plant (Pierre and Stuart (1933), Wright (1937)).

However, the mechanism of the aluminium-phosphate relationship within the plant appears to be still obscure. The hypothesis that the precipitation of phosphorus within the plant due to a combination with aluminium results in a deficiency of phosphorus, does not appear to be feasible, in view of the fact that the amount of aluminium present is only a fraction of the phosporus content of the plant. On the other hand, it may be that the location of aluminium in the tissues of the roots, as indicated by the microchemical tests described above, may eventually lead to some reduction in the intake of phosphate by the roots, and interfere with its translocation within the plant.

From the work of Magistad (1925), it seems probable that aluminium displaces iron. He found that plants supplied with aluminium showed an increased percentage of this element in the tissues, while that of iron was reduced.

Results of the Burnley experiments further showed that aluminium had a marked effect in preventing or delaying the development of toxicity symptoms (particularly iron deficiency chlorosis) resulting from the addition of excessive quantities of either manganese or zinc to the solution (Pl. II, fig. 2). This is described further in the sections relating to the effects of excesses of manganese and zinc respectively. It seems probable that this detoxifying effect of aluminium is related to its influence on the utilization of phosphorus in the plant as described above. Somers and Shive (1942) (but see Leeper (1944)) have suggested that the effect of excess manganese (or cobalt) is to catalyze the oxidation of the iron to the ferric state, and to precipitate it in the form of some ferric-phosphate-organic complex, thus inducing iron deficiency. It is evident, therefore, that if the phosphorus were made unavailable to plant metabolism by aluminium, this ferric-phosphate complex could not be formed, so that the plant would not suffer from iron deficiency in the presence of excess manganese or zinc. That such an explanation is possible is indicated by the fact that plants subjected to phosphate deficiency, together with an excess of either manganese or zinc in the nutrient solution, manifest considerably less severe symptoms of iron deficiency chlorosis compared with those produced in plants receiving the same excess of manganese or zinc but a normal level of phosphorus (Pl. II, fig. 4).

Haas (1936a), Schappele (1945) and Liebig *et al.* (1942) also observed that aluminium exhibited a similar detoxifying effect with respect to zinc, manganese and copper respectively. The latter's results indicated that the detoxifying influence of aluminium on copper was seated in the roots. The aluminium did not prevent the absorption of copper by the roots, but did in some manner prevent injury by this element. Liebig *et al.* conclude that 'the antagonistic effect of a trace of one element upon another may profoundly influence plant growth, and yet mean nothing with regard to essentiality.'

Excess calcium added in conjunction with 25 p.p.m. of aluminium tended to offset slightly the harmful effect of the excess aluminium. This accords with the results of Eisenmenger (1935), who found that the calcium ion was a decided factor in overcoming the toxicity of aluminium.

Excess Copper

At a given concentration, copper was found to be much more toxic to flax than zinc. De Rose *et al.* (1937) obtained a similar result with tomato plants. As little as 0·5 p.p.m. of copper induced severe symptoms in flax, while with more than 2 p.p.m. practically no growth occurred. On the other hand, at a concentration of 0·05 p.p.m. of copper the growth of the flax was practically normal. With oats Piper (1942) observed an initial depression in growth with more than 0·25 p.p.m. of copper. Later, however, even plants receiving 3 p.p.m. of copper showed considerable recovery. It would thus appear that oats are more tolerant to high concentrations of copper than flax.

The effects of copper toxicity in flax were first manifest by a retardation of growth, and the development of a chlorosis and necrosis of the top of the plant (Plate II, fig. 3). This chlorosis was prevented by supplying extra iron to the solution. The relation of this copper-induced iron deficiency to molybdenum has been described by Millikan (1947c). References to copper-induced chlorosis in plants have been given by Wallace and Hewitt (1946) and Millikan (1947c).

A secondary symptom of copper toxicity consisted of a necrosis of the lower leaves (which were usually chlorotic), commencing from the tips. This lower leaf necrosis also occurred in plants receiving excess copper together with additional iron to alleviate the iron deficiency chlorosis. Broyer and Furnstall (1945) also describe a progressive death of the older leaves of barley plants from the tip due to copper toxicity.

Copper in the solution was much more toxic to the root development of flax than was zinc or manganese (Pl. III, fig. 1). The copper-poisoned roots were brown, very stunted, and without any laterals. Similarly, Broyer and Furnstall found that the root growth of barley was particularly affected by the presence of small amounts of copper in the solution.

Excess Zinc

The addition of as little as 5 p.p.m. of zinc to the normal nutrient solution had a deleterious effect on growth. The first symptoms produced by an excess of zinc consisted of iron deficiency chlorosis (Pl. II, figs. 3, 4; Pl. III, fig. 1). This aspect of zinc poisoning has been described by Wallace and Hewitt (1946) and Millikan (1947b). It was possible to prevent entirely the development of this chlorosis of the top of the plant by supplying additional iron to the solution at the same time as the excess zinc was added. However, although the additional iron prevented the development if iron deficiency chlorosis, it did not alleviate other toxic effects of zinc such as dwarfing of the roots and aerial parts of the plant (Pl. II, fig. 4).

The appearance of iron deficiency chlorosis was also considerably delayed, and its severity much reduced, by subjecting the plants to phosphorus deficiency in conjunction with excess zinc, although the plants remained dwarfed. This result is further discussed in the section on excess aluminium. By contrast, the chlorosis associated with excess zinc was aggravated by adding excess phosphate (0·005M or 0·010M

$NaH_2PO_4.12H_2O$) to the excess zinc solutions (Pl. II, fig. 4). The same excess of phosphate in the presence of a normal zinc concentration also induced iron deficiency chlorosis.

However, excess phosphate (in the presence of additional iron to prevent iron deficiency chlorosis in the plants) greatly retarded the development of the lower leaf necrotic symptoms described below as being associated with excess zinc in the nutrient solution. This supports the existence of a zinc-phosphorus relationship in the plant (Millikan, 1946, 1947a). The severity of zinc-induced chlorosis was found to be dependent upon the supply of molybdenum (Millikan, 1947c).

A feature of the effect of excess zinc in inducing iron deficiency was that the chlorosis was less marked in the presence of 100 p.p.m. of zinc than 10 or 20 p.p.m. However, with the increasing concentrations of zinc, growth was progressively reduced, so that the lack of chlorosis at the higher zinc concentrations may possibly be attributable to a lower iron requirement due to the poorer growth. On the other hand, the control solutions, which received the same amount of iron, made the best growth and showed no chlorosis.

The secondary symptoms produced by excess zinc occurred on the older leaves of the plant, and consisted of a necrosis which commenced at the tips of the lower leaves. These symptoms have been described by Millikan (1947b). The necrosis of the tip of the leaf was usually associated with a slight chlorosis of the non-necrotic portion of the leaf (Pl. III, fig. 2). This serves to distinguish this necrosis from the lower leaf necrosis caused by excess manganese. However, the lower leaves of plants receiving excess zinc in the nutrient solution often showed another characteristic symptom in the form of numerous small bronze to brown coloured spots which usually, but not always, occurred first on the under surface of the leaf. In one experiment the spots were first discernible on the upper surface. Later, however, the spots are discernible on both surfaces, coalesce, and become necrotic.

The leaf necrotic symptoms referred to above were identical, irrespective of whether or not the iron deficiency chlorosis was prevented by supplying extra iron to the solution.

Plants receiving excess zinc sometimes developed numerous necrotic spots on the lower portion of the stems. Individual spots were light to rusty brown in colour, and were larger than those caused by excess manganese.

It was found that a concentration of 0·0005M $AlCl_3.6H_2O$ in the culture solution had the effect of markedly alleviating the toxic effects of concentrations of 10, 20, 50 or 100 p.p.m. of zinc (Pl. II, fig. 2). Little initial iron deficiency chlorosis developed, and the onset of leaf necrosis was considerably delayed, although when it did occur its characteristics were similar to those described above. A possible explanation of this effect is submitted in the section on excess aluminium. Haas (1936a) and Schappele (1945) have also reported that where zinc concentrations in the culture solution became somewhat excessive, the addition of aluminium benefited growth. Schappele also reported that the addition of boron counteracted the toxic effects of zinc.

Root development of the flax plants grown in the presence of excessive amounts of zinc was severely curtailed. In this respect, excess zinc was more toxic than excess manganese, but less toxic than excess copper (Pl. III, fig. 1).

Excess Manganese

Variable leaf characteristics resulting from an excess of manganese in the nutrient solution have been obtained in different experiments. The cause of this variation has not been fully established, although it appeared to be partly related to seasonal conditions. In this regard, Hopkins *et al.* (1944) have demonstrated that light intensity may have an important effect on the type of symptom induced by excess manganese, while Gile (1916) has reported manganese chlorosis (which is due to iron deficiency) as being most intense during winter months. Chapman (1945) has also observed that more iron chlorosis occurred in his citrus cultures in winter than in summer.

In a solution of pH 7, the young flax plants appeared to tolerate a concentration of 10 p.p.m. of manganese without any harmful effects on growth becoming apparent for several weeks at least. When the seedlings were subjected to a greater excess of manganese (25, 50, 100 or 150 p.p.m. respectively) they showed signs of toxicity symptoms after approximately 12 to 14 days.

There was evidence that the minimum toxic concentration of manganese was lower in the solution at pH 4·5 than in that at pH 7·0. This accords with the results of Olsen (1934, 1936) and Chapman *et al.* (1939), who showed that plants were able to absorb more manganese in an acid solution than in a neutral solution.

The tops of the plants developed a chlorosis (Pl. II, fig. 3; Pl. III, fig. 1), while in the neutral solution a brown necrosis developed at the middle of one or both edges of the second lowest pair of leaves. This necrosis soon involved the whole of the distal half of the leaf. In the solution of pH 4·5 the flax seedlings did not develop this 'middle leaf necrosis' of the second pair of leaves. However, the subsequent symptoms described below were identical for both solutions.

It was found that the intensity of the chlorosis of the top of the plant referred to above could be reduced, or its appearance prevented, by the addition of extra iron to the solution. However, the addition of extra iron to the solution did not prevent the occurrence of other toxic symptoms due to excess manganese, e.g., dwarfing and lower leaf necrosis. The chlorosis appeared to be more severe in experiments conducted in winter than in summer.

The literature on manganese-iron relationships in plants has been reviewed by Twyman (1946), Wallace and Hewitt (1946), and Millikan (1947). It has been claimed by Somers and Shive (1942) that pathological symptoms resulting from excess manganese are identical with those of iron deficiency and vice versa. This conclusion is not supported by the results of the Burnley water culture experiments, where, as described below, a characteristic type of necrosis, not typical of iron deficiency, occurred consistently in the leaves of flax plants receiving excess manganese (Pl. IV, figs. 1, 2) even when the iron

deficiency symptoms had been remedied or prevented by the administration of additional iron (2, 4, 5 or 10 times the normal concentration) to the solution. The plants so treated actually showed a condition of hyperchlorophylly, and were still dwarfed. The characteristics of the leaf necrosis were similar at all levels of iron. Hopkins *et al.* (1944) also found that when the iron deficiency chlorosis in pineapple plants growing on a manganiferous soil had been prevented by spraying with iron sulphate, the plants still continued to absorb large amounts of manganese, and developed other peculiarities. Further, the symptoms induced in flax by an excess of iron in the Burnley water cultures were not those of manganese deficiency chlorosis. Again, it has been shown that iron deficiency chlorosis in plants may also be induced by an excess of copper, zinc, cobalt or nickel as well as by excess manganese, and that its severity in all these instances is dependent upon the supply of molybdenum (Millikan, 1947c). The supply of aluminium will also affect manganese-induced iron deficiency chlorosis.

In the water cultures, the general growth of the flax plants receiving excess manganese became retarded, and within a fortnight of setting up, and at the higher manganese concentrations, lower leaves other than the second pair of leaves commenced to become necrotic from the tips downwards. In some leaves the necrosis first appeared not at the ultimate tip, but very close to it, in the form of minute dark spots on the edges of the leaves. These necrotic spots soon enlarged until the whole of the top half of the leaf was involved. It was characteristic of this type of leaf symptom that the spread of the necrosis did not involve the lower half of the leaf, which remained practically normal green in colour (Pl. IV, fig. 1). When they first appear, the necrotic spots may be seen on both surfaces of the leaf, although they may be more pronounced on the upper surface. This serves to distinguish them from early zinc toxicity symptoms.

At first the necrotic tips were usually brown to dark brown, later becoming lighter in colour and the plants as a whole showed only a slight general chlorosis.

Another type of leaf symptom associated with an excess of manganese in the nutrient solution consisted of numerous necrotic spots on the lower leaves. These spots were characteristically dark brown and occurred on any portion of the leaf, but were largest at the sides and tip (Pl. IV, fig. 2). They occurred simultaneously on both surfaces of the leaf. The leaves later became somewhat distorted, being bent and slightly twisted. This necrotic spotting has occurred with and without much chlorosis, depending on the level of iron in the nutrient solution. The factors governing the occurrence of this type of leaf spotting rather than tip necrosis, have not been determined. A further symptom of manganese toxicity in flax was the occurrence of numerous small brown spots on the stems of the plant. These spots often coalesced to form larger browned areas.

It was found that the addition of aluminium chloride 0·0005M, or $AlK(SO_4)_2.12H_2O$ at a concentration of 1 p.p.m. of aluminium, to culture solutions containing excess manganese (up to 150 p.p.m.), considerably reduced the intensity of, or entirely eliminated, the chlorosis

and retarded the development of leaf necrosis (usually tip necrosis). Schappele (1945) also found that the addition of aluminium (or boron) counteracted the chlorosis associated with manganese toxicity in pineapples grown in water cultures.

Bortner (1935) has reported that the removal of phosphorus from the culture solution lowered the manganese concentration at which iron deficiency chlorosis developed. This effect could not be confirmed in the Burnley water culture experiments. Actually, it was found that phosphorus deficiency in conjunction with excess manganese (10, 25, 50, 100 and 150 p.p.m. respectively) prevented the development of iron deficiency chlorosis. This effect was considered to be due to the fact that phosphorus-deficient flax plants are typically darker green than the normal (Millikan, 1944) and evidently contain a higher content of physiologically active iron than plants grown at a higher phosphate level. Excess manganese (100-150 p.p.m.) in conjunction with phosphorus deficiency also did not cause such a marked dwarfing of the flax plants as when the same excess treatment was applied with a normal solution. However, leaf necrotic symptoms due to the excess of manganese were identical in the presence of either a normal or deficient supply of phosphorus.

On the other hand, it was found that the addition of excess phosphate (0·005M or 0·010M NaH_2PO_4) to cultures containing excess manganese increased the severity of iron deficiency symptoms to such a degree that the tops of the plants soon became necrotic.

It seems evident from the results obtained with tobacco by Swanback (1939) that an antagonism exists between calcium and manganese in their absorption. Increasing calcium concentrations were found to reduce the intake of manganese and its translocation to the leaves. Conversely, Fried and Peech (1946) found that excessive manganese absorption reduced the uptake of calcium.

Excess Cobalt

Within three days, the lateral root growth of plants in the solutions receiving 1 p.p.m. or more of cobalt was noticeably stunted, whereas in the cultures receiving 0·5 p.p.m. of cobalt no such stunting occurred.

The tops of the plants became dwarfed and soon developed a chlorosis which was characteristic in that the main veins of the leaves remained green (Pl. III, fig. 3). This was in contrast with the chlorosis caused by excess copper, zinc, manganese and nickel, where the whole of the leaf became yellow or even white. The severity of the cobalt-induced chlorosis was reduced by supplying additional iron to the solution. Brenchley (1938) and Somers and Shive (1942) have shown that excess cobalt will produce symptoms similar to, or identical with, iron deficiency chlorosis in plants.

In the case of flax, the chlorosis of the upper leaves was followed by a necrosis of the top of the plant. With 5 p.p.m. of cobalt, this necrosis first appeared within 14 days of setting up the cultures. Aerial growth at this concentration was very severely reduced.

A characteristic type of necrosis also appeared in the older leaves. It commenced on one or both edges of the lower half of the leaf. It thus

differed from the lower leaf tip necrosis induced by excess manganese, zinc or copper. The severity of cobalt-induced chlorosis and necrosis was reduced by supplying additional molybdenum to the solution (Millikan, 1947c).

Excess Nickel

A concentration of 0·5 p.p.m. of nickel caused a noticeable retardation in lateral root development. This retardation became more severe with increasing concentration. With 5 p.p.m. of nickel, little aerial growth occurred and the tops of the plants finally became necrotic. Chlorosis of the top leaves occurred at all concentrations of nickel used. The severity of this chlorosis was reduced by supplying extra iron or molybdenum to the solution (Millikan, 1947c). Haselhoff (1893), Cotton (1930) and Brenchley (1938) report that nickel toxicity symptoms consist of a chlorosis of the youngest leaves.

Summary

During 1943 to 1947 inclusive, many water culture experiments have been made to determine the symptoms produced in flax by excesses of boron, iron, molybdenum, aluminium, copper, zinc, manganese, nickel and cobalt.

Excess boron resulted in the death of the older leaves of the plant. No chlorosis occurred.

Excess iron induced a hyperchlorophylly. Growth of tops and roots was stunted. The thickened roots contained abundant inorganically bound phosphorus. Phosphorus deficiency intensified the hyperchlorophylly and excess phosphorus reduced it.

Excess molybdenum caused a stunting of roots and tops and a golden yellow to orange colouration. The presence of tannins was detected in the discoloured tissues.

Excess aluminium caused the roots to be dwarfed and thickened, with very little lateral growth at the higher concentrations. Top growth was also dwarfed and darker green than normal. The presence of aluminium was detected in the cortical and meristematic tissues of the poisoned roots. Aluminium had a marked effect in reducing or preventing the occurrence of iron deficiency chlorosis caused by excess zinc or manganese.

Excess copper was very toxic to flax. It induced iron deficiency chlorosis, also necrosis of the lower leaves from the tips.

Excess zinc caused severe dwarfing and iron deficiency chlorosis, also necrosis. The severity of this chlorosis was reduced by additional iron or by phosphorus deficiency. It was aggravated by excess phosphorus. Necrosis from the tips of the lower leaves occurred. Minute quantities of aluminium alleviated the toxic effects of excess zinc. Root development was severely restricted. Zinc was more toxic than manganese, but less than copper in this respect.

Excess manganese induced iron deficiency chlorosis, which was prevented or cured by supplying additional iron, molybdenum or aluminium, or by phosphorus deficiency. Secondary symptoms consisted of a tip necrosis or a necrotic spotting of the lower leaves.

Excess nickel and excess cobalt both induced iron deficiency chlorosis, also necrosis in flax, the severity of which was reduced by applying small quantities of molybdenum.

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Description of Plates

Plate II

Fig. 1.—Results of microchemical tests for the presence of aluminium in the roots of flax plants grown in solutions containing excess aluminium, (a) using aurine tricarboxylic acid, (b) and (c) using haematoxylon. The presence of aluminium in the cortical and meristematic tissues is indicated.

Fig. 2.—Showing the marked effect of aluminium in delaying the appearance of toxicity symptoms normally resulting from the presence of excess zinc in the nutrient solution.

Fig. 3.—Showing the iron deficiency chlorosis and necrosis in flax resulting from excess of zinc, manganese and copper in the nutrient solution. Left to right: Normal solution; zinc, 10 p.p.m.; manganese, 10 p.p.m.; copper, 2 p.p.m.

Fig. 4.—Changes in reaction to excess zinc in the nutrient solution caused by changes in the amount of phosphorus and iron present. Left to right: (i) Normal solution. (ii) Nutrient solution containing excess zinc, 20 p.p.m.: iron deficiency chlorosis. (iii) Same excess of zinc and phosphorus deficiency: no iron deficiency chlorosis. (iv) Same excess of zinc and iron excess: iron deficiency chlorosis completely prevented; hyperchlorophylly. (v) Same excess of zinc and excess phosphorus: more severe iron deficiency chlorosis.

Plate III

Fig. 1.—Effects of excesses of manganese, zinc and copper on root development of flax grown in water cultures. Left to right: Normal solution; excess manganese, 50 p.p.m.; excess zinc, 50 p.p.m.; excess copper, 2 p.p.m. The plants subjected to excess all showed iron deficiency chlorosis.

Fig. 2.—Lower leaf necrosis caused by excess zinc. The non-necrotic portions of the leaves are slightly chlorotic.

Fig. 3.—Cohalt toxicity symptoms. The main veins of the leaves remain green while the rest of the leaf shows iron deficiency chlorosis. Necrosis of the lower leaves usually commences at the bases of the leaves.

Plate IV

Fig. 1.—(a) and (b)—Necrotic symptoms in the lower leaves of flax seedlings caused by excess manganese (100 p.p.m.). The necrosis commenced at or near the tips and gradually extended, while the non-necrotic portions of the leaves remained a normal green colour.

Fig. 2.—Leaf 'spotting' symptoms produced in flax grown in a nutrient solution containing excess manganese.